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ENVIRONMENTAL MEDICINE GENOME BANK (EMGB): CURRENT COMPOSITION

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on the use of volunteers in research.

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The USARIEM Environmental Medicine Genome Bank (EMGB) project is an ongoing effort to identify and characterize genes relevant to environmental injuries and illnesses and to human physical performance. To accomplish this, the EMGB banks DNA samples from human volunteers who have participated in USARIEM environmental and human performance studies and maintains a registry of phenotypic information. Because of the ethnically diverse and geographically dispersed backgrounds of the donors, the EMGB can be used to identify polymorphisms in genes that are potentially of interest to environmental medicine and to obtain an estimate of the frequency of these polymorphisms in young, healthy U.S. adults. Additionally, this resource also serves as a valuable source of control material for genetic studies of human diseases, such as asthma. The project is performed as part of a cooperative research and development agreement (CRDA) with the Division of Pulmonary and Critical Care Medicine at Brigham and Women's Hospital. This report provides updated information about the samples currently stored in the EMGB. It is intended as a reference document for researchers who wish to make use of this resource, and fulfills the annual reporting requirement of CRDA number DAMD 17-00-0017.

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EXECUTIVE SUMMARY

The USARIEM Environmental Medicine Genome Bank (EMGB) project is an ongoing effort to identify and characterize genes relevant to environmental injuries and illnesses and to human physical performance. To accomplish this, the EMGB banks DNA samples from human volunteers who have participated in USARIEM environmental and human performance studies and maintains a registry of phenotypic information. Because of the ethnically diverse and geographically dispersed backgrounds of the donors, the EMGB can be used to identify polymorphisms in genes that are potentially of interest to environmental medicine and to obtain an estimate of the frequency of these polymorphisms in young, healthy U.S. adults. Additionally, this resource also serves as a valuable source of control material for genetic studies of human diseases, such as asthma. The project is performed as part of a cooperative research and development agreement (CRDA) with the Division of Pulmonary and Critical Care Medicine at Brigham and Women's Hospital.

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INTRODUCTION

Based on recent reports, it seems likely that there is a significant genetic contribution to some aspects of human physical performance (1-5, 7, 8) and to the susceptibility to environmental illness and injury. However, very few candidate genes have been identified, in part because few laboratories have access to large populations of well-characterized subjects drawn from a wide variety of genetic backgrounds. The U.S. Army Research Institute of Environmental Medicine (USARIEM) is uniquely qualified to undertake a search for these genes, by virtue of its access to Army personnel and its ability to define precisely those phenotypes relevant to environmental illnesses and human performance.

Large numbers of samples are typically needed to identify genes that contribute to complex traits (2). Accordingly, the USARIEM Environmental Medicine Genome Bank (EMGB) banks DNA samples (obtained from donor white blood cells) and catalogues phenotypic information obtained over the course of multiple USARIEM studies. By pooling samples and data from several studies, it becomes possible to undertake genetic analyses that would otherwise not be feasible.

The EMGB serves as an Institute resource, and anonymous aliquots from the bank are available to individual investigators upon request. This document summarizes the current contents of the bank.

MATERIALS AND METHODS

VOLUNTEERS

With the exception of study #1 (normal controls), subjects were participants in other USARIEM studies of environmental medicine and physical performance (Table 1). All subjects gave consent in accordance with Army Regulation 70-25. In study #1, subjects were recruited directly for the purpose of creating a core cohort of DNA samples from anonymous, young, otherwise healthy volunteers.

20 ml of blood were drawn from each volunteer into 10 ml, heparin-containing tubes. Samples drawn from locations other than USARIEM were shipped overnight (at room temperature) to the laboratory for processing.

DNA ISOLATION AND STORAGE

DNA is obtained from leukocyte nuclei after erythrocyte lysis, using the QIAamp Maxi Kit (Qiagen, Inc., Santa Clara, CA). The isolated DNA is stored in aqueous solution (in water), at a concentration of 35-150 ng / µl (determined from UV absorption at 260 nm).

To minimize damage from repeated freeze-thaw cycles, each sample is divided into a master sample and several aliquots at the time of original isolation. At present, all samples are maintained at -80°C. Aliquots are used until exhausted. The master samples are thawed only when new aliquots are needed.

RESULTS

CONTRIBUTING STUDIES AND SAMPLE USE

Studies that have contributed samples to the EMGB and the current inventory of samples are listed in Table 1. This table also lists some of the phenotypic information available and summarizes some of the genotypic information that has been obtained on the samples to date. So far, six USARIEM studies have contributed samples to the EMGB. DNA was obtained from most (but not all) donated samples, and some samples (especially those with low DNA yields) have been used in their entirety. At present, the bank contains DNA samples from 272 different donors.

Samples from the EMGB have been used in three genetic studies to date: (a) The Angiotensin Converting Enzyme I/D polymorphism in intron 16 has been implicated by some as a marker of physical performance (5, 7, 8), though others have questioned this association (10, 11). We are presently investigating whether this polymorphism is associated with physical performance responses to basic training. (b) The neuronal nitric oxide synthase (NOS1) CA repeat polymorphism in exon 29 has been found to be associated with asthma, in a study to which the EMGB contributed samples (6). (c) The EMGB was used recently to determine the frequency of a novel mutation in the eotaxin gene that limits eotaxin secretion (9).

Table 1. Summary of the Contents of the EMBG.

Study #	Study Designation	PI, Division	Study Location	Study Dates	Samples Submitted	Samples Currently Banked	Phenotypic Information	Genotypes Studied
1	Normal Controls	Sonna, TMD	USARIEM	Mar - Apr 1998	62	57	1,2	ACE, EOT
2	H98-07 Physical Fitness of Soldiers Entering and Completing Basic Combat Training and its Role in Injury Incidence	Sharp, MPD	Ft. Jackson, South Carolina	Jun - Jul 1998	152	146	1,5,6,7,9,10	ACE, NOS1, EOT
3	H97-10 Warfighter Physiologic Status Monitoring: Body Core Temperature, Blood Oxygen Saturation and Environmental Symptoms during an Expedition to Mt. Logan, Canada	Muza, TMD	Mt. Logan, Canada	May - Jun 1999	13	13	1,2,3,4,9	ACE, EOT
4	H98-09 Effect of Residence at Low and Moderate Altitudes on Arterial Oxygen Saturation at Moderate-to-High Altitudes	Muza, TMD	Pike's Peak, Colorado	Jun 1999	40	40	1,2,4,8,9	EOT
5	H99-12/A-9212 Role of Exercise During Intermittent Exposures to Hypobaric Hypoxia on Acclimation to 4300 m	Beidleman, TMD	USARIEM	Oct 1999	8	8*	1,2,4	EOT
6	H99-03 Role of Leukotrienes in High Altitude Illness	Muza, TMD	USARIEM	Jan - Feb 2000	9	8*	1,2,9	

*(One subject participated in both studies and is only counted once for purposes of the EMGB).

Table 1 (Continued)

Key to Available Phenotypic Information

1. Age, race and gender
2. Smoking status
3. History of asthma or exercise-induced bronchospasm
4. Spirometry data
5. Spirometry before and after exercise
6. Army Physical Fitness Test scores
7. Army Physical Fitness Test (APFT) scores before and after basic training
8. Oxygen saturation with increasing altitude
9. Height and weight

Key to Genotypes

ACE: Angiotensin Converting Enzyme Insertion/Deletion Polymorphism, Intron 16
NOS1: Neuronal Nitric Oxide Synthase CA repeat Polymorphism, Exon 29
EOT: Eotaxin 23ALA → 23 THR Polymorphism

DEMOGRAPHIC INFORMATION

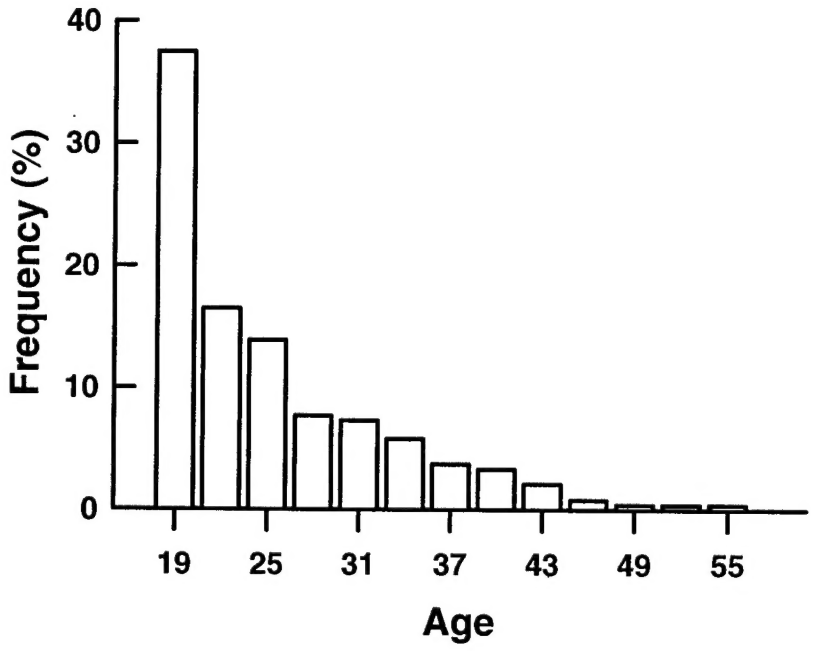
A summary of the ages, races and genders of the subjects for whom current samples exist in the EMGB is given in Table 2. The distribution of the ages of the donors at the time of sample collection is illustrated in Figure 1. The median age of the subjects who have donated to the EMGB is 23 (interquartile range, 19.25-29). Slightly more than half (56%) of the subjects are male. About a third of the samples were donated by subjects of ethnic backgrounds other than Caucasian; 19% of the samples were donated by subjects of African-American origin. Homes of origin are known for 71% of the subjects, and include 44 different U.S. states, two U.S. territories, and two foreign nations.

Table 2. Demographic Characteristics of the EMGB.

Demographic	N =	%
Gender		
Male	152	56%
Female	120	44%
Ethnic Origin		
Asian	11	4%
African	52	19%
Caucasian	182	67%
Hispanic	24	9%
Native American	2	<1%
Other	1	<1%

	Median	Interquartile Range
Age	23	19.25 – 29

Figure 1. Distribution of the ages (at the time of donation) of subjects who have donated samples to the EMGB. The histogram only includes samples that are currently available.



DISCUSSION

The EMGB consists of DNA samples obtained from ethnically diverse and geographically dispersed donors. This diversity makes the EMGB a valuable resource for several types of genetic studies. At present, we envision three principal uses for the bank. First, given a gene known or suspected to be of interest to environmental medicine, the EMGB can be used to identify new polymorphisms in this gene and to obtain an estimate of the frequency of these polymorphisms in young, healthy U.S. adults. Because the information collected in the EMGB includes both ethnic origin and gender, it is also possible to compare allele frequencies across important demographic subgroups. Second, the EMGB is an excellent source of control material for genetic studies of human diseases, such as asthma. Third, some of the donor phenotypes in the EMGB (particularly those from study #2) are well characterized enough to allow genetic association studies. An example of one such study is our ongoing effort to determine whether there is a clinically important association between ACE I/D genotype and the physical performance response to basic training.

At present, one significant limitation of the EMGB is that samples are not easily renewed, as they are stored as extracted DNA in solution. Once exhausted, the only way to renew a sample is to ask the volunteer for another donation. One way to overcome this problem is to immortalize lymphocytes obtained from each volunteer and use the cell lines thus created as a renewable source of DNA. Accordingly, we are in the process of adopting existing techniques for lymphocyte immortalization (12) to our laboratory.

In summary, the current heterogeneity of the EMGB makes it a valuable resource for genetic research. It has already proven to be of value in collaborative studies of human disease (6, 9) and is being used to examine the genetic basis of physical performance. The anticipated addition of infinitely renewable sources of DNA to the collection will greatly enhance its value.

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